Application of hybridoma technology to problems in the agricultural sciences

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Abstract The development of methods for the production of monoclonal antibodies has provided a powerful tool for virtually all areas of the biological sciences. Monoclonal antibodies allow the dissection of complex mixtures mixtures of antigenic molecules; by applying screening procedures to a library of monoclonals, selection for properties such as viral neutralization or enzyme inactivation is possible, thereby identifying proteins with specific biological activities. Similarly, monoclonal antibodies can be used to map the surface of a protein, define cell surface antigens, and localize molecules within cells or tissues. The property of unique specificity associated with clonally-derived antibodies has served to revolutionize the field of diagnostics where the use of polyclonal antisera once dominated. The use of monoclonal antibodies for antigen identification has greatly benefited the study of the immune response to infectious agents and the development of vaccines. With the advent of better methods for the generation of hybridomas such as electrofusion and oncogene transfection, modifications allowing the exploitation of non-immunogenic molecules, and the development of techniques for producing interspecific hybrids, the contribution of this technology as a tool for research in the agricultural sciences will undoubtedly increase.

Introduction

In the decade since the development of methods for the production of antibody-secreting hybrid cell lines (Kohler and Milstein, 1975) this technology has had major impact in virtually all areas of the biological sciences. The reason for the tremendous application of hybridoma technology lies in the properties of the monoclonal antibody secreting hybrid cells derived from the parent cell lines, namely the antibody secretion of the lymphocyte and the immortality of the myeloma.

Stimulation of the immune system by an antigen or mixture of antigens results in the proliferation of populations of B-lymphocytes which produce antibodies with specificities for an array of antigenic determinants. The formation of hybrids by fusing these lymphocytes with myeloma cells results in populations of immortalized antibody secreting cells. The subsequent cloning of these hybrid cells results in the establishment of unique cell lines, each derived from a single B-cell and myeloma parent, capable of growing continuously in culture and secreting an antibody with a single molecular configuration (heavy and light chain) and variable region antigen specificity.

P. C. Augustine et al. (eds.), *Biotechnology for Solving Agricultural Problems*

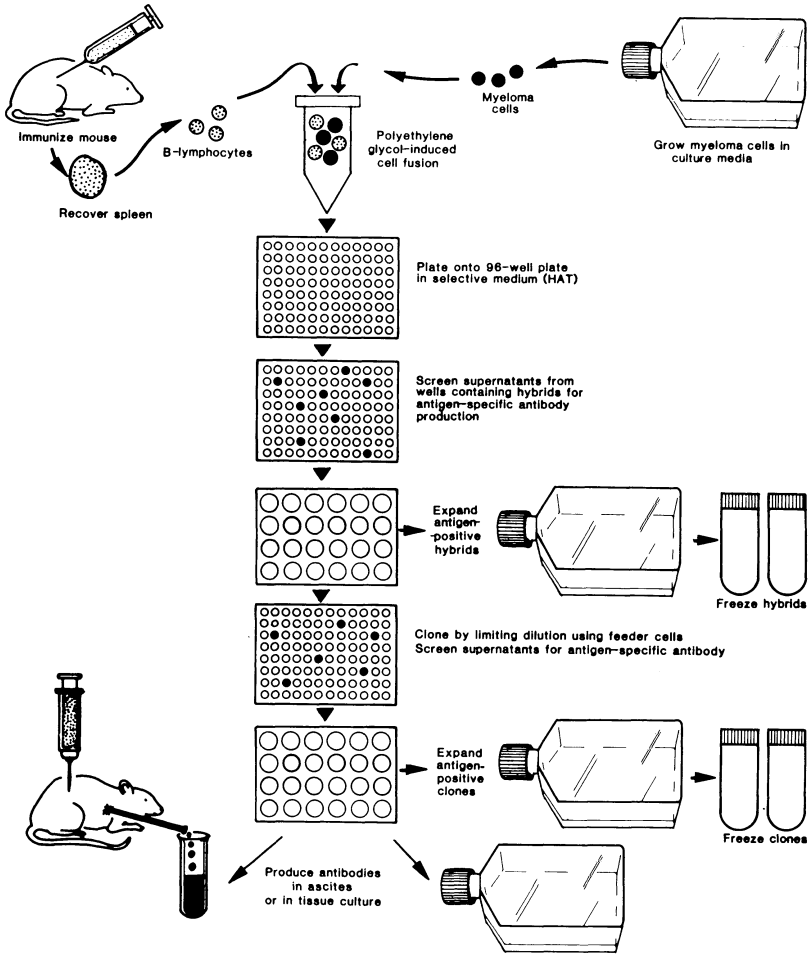

Figure 1. General methods for the production of monoclonal antibodies.

Current technology

The methods employed in the production of antibody-secreting hybrid cell lines are relatively simple, having their basis in somatic cell hybridization (see Goldsby, Srikumaran and Guidry, 1984, for review). Conventional procedures for the production of monoclonal antibodies are detailed in Fig. 1. Appropriate immunization protocols are often determined empirically, however, higher affinity antibodies are usually generated with an increasing number of immunizing doses. It is often useful to check serum for the appropriate specificity prior to fusion. An intravenous boost 3–4 days prior to fusion is generally recommended.

Spleen cells and myeloma cells are normally fused in the presence of polyethylene glycol, however, many modifications and alternative methods exist (Oi and Herzenberg, 1980; Galfre and Milstein, 1981). Following fusion hybrid cells are selected from unfused myeloma cells by growth in selective media (hypoxanthine, aminopterin, thymidine; Littlefield, 1964). The myeloma cells, with a deficiency for a purine salvage pathway enzyme (hypoxanthine phosphoribosyl transferase), are selected against by growth in inhibitors of de novo purine synthesis (aminopterin, amethopterin). Hybrids containing the HPRT gene donated from the spleen cell partner can grow with exogenous hypoxanthine; thymidine is also added since aminopterin inhibits the formation of thymidylate.

Once hybrids have been generated, it is of extreme importance to apply selective screening procedures; subsequent expansion, cloning, cryopreserving, freezing, and antibody production procedures are laborious so it is counterproductive to retain hybrids of no value. A variety of screening procedures are available for selecting hybrid cell lines of interest (Gamble, 1984a). Screening may be tailored to select not only for antigen specificity, but also for antibodies with specific properties such as complement fixations, binding to Staph protein A, or hemagglutination. Selected hybrids should be cloned (McKearn, 1980) as soon as possible, and cryopreserved (Kennett, 1980). Recloning occasionally is necessary to eliminate the subpopulations of hybrids which have stopped secreting.

Preliminary biochemical characterization of monoclonal antibodies may provide useful information. Such characterization often includes determination of antibody isotype by gel diffusion (Letchworth and Appleton, 1984), metabolic labeling (Haas and Kennett, 1980) or isotype specific ELISA (Gamble, 1984a), and the determination of antibody specificity by immunoblotting (Lampson and Fisher, 1985) or immunoprecipitation (Lampson, 1980).

New developments in monoclonal antibody technology

A number of modifications in hybridoma technology have both facilitated the production of hybrids and the recovery of their antibody products.

In vitro immunization

Novel methods have been developed for the immunization of donor B lymphocytes in vitro (Borrebaeck, 1984; McHugh, 1984). In vitro immunization is particularly useful in situations where only small amounts of antigen are available, when antigens are toxic to animals, or

when hybrids using human cells are being developed. For in vitro immunization, lymphocytes, grown in medium supplemented with thymocyte growth factors, are exposed to low concentrations of antigen (VanNess, Laemmli , and Pettijohn, 1984). Antigen exposure may be primary or secondary following an initial in vivo stimulation. Blast transformation in response to antigen is then observed as clusters of dividing cells; blast transformed lymphocytes are then used as fusion partners with appropriate myeloma cells.

Fusion technology

Hybridoma technology in the study of immunoglobulins and the immune response to antigens in animals of agricultural and veterinary interest has been hindered by the lack of identified myeloma fusion partners for lymphocytes from these species. However, recent studies have demonstrated the potential of interspecific hybridomas, produced by fusing mouse myeloma cells with bovine lymphocytes (Srikumaran, Guidry and Goldsby, 1983). These studies have shown that although the frequency of stable hybrids is low, due to chromosome loss, rigorous selection allows for the stabilization of some cell lines. Initial studies have used hybridoma secreted monoclonal bovine immunoglobulins to biochemically and immunochemically characterize these immunoglobulin molecules, however, recent studies have generated mouse/bovine hybrids with specificity for a particular pathogen (Raybould et al., 1985). Application of interspecific fusion techniques to other livestock species is possible and should greatly facilitate the study of the immune response in these species to specific pathogens.

Other new methods have been developed for the fusion or immortalization of lymphocytes. One example is the technology of electrofusion (Zimmerman and Vienkin, 1984). Electrical fusion of cells relies on a reversible electrical breakdown of cell membranes in response to high intensity, short duration electrical pulses. Cells to be fused are aligned and allowed to establish membrane contact in an alternating current electrical field. Fusion is then accomplished by applying pulses of direct current of sufficient strength to cause a reversible breakdown in the integrity of the cell membranes. Production of both mouse and human hybridomas by electrofusion has been described. The application of electrical fusion is particularly appropriate if preselection of lymphocytes used for fusion is possible (Zimmerman and Vienken, 1984).

An extremely promising technology for producing immortalized antibody-secreting, as well as other types of cell lines, is transfection. Transfection involves the incorporation of oncogenic DNA into the chromosomes of normal cells, in this case, B lymphocytes, by various

methods including calcium phosphate precipitation or electrical pulse mediated cell breakdown as described for electrofusion. Genomic DNA, extracted from myeloma cells, has been incorporated into mouse and pig lymphocytes, resulting in monoclonal antibody secreting cell lines (Jonak et al., 1984; Davis et al., 1985). The application of transfection technology in the veterinary sciences will facilitate the production of antibody producing cell lines for large animal species. With the identification of specific oncogenic DNA sequences and incorporation of these sequences into shuttle vectors the possibilities exist for the immortalization of a wide variety of cell lines.

Antibody production

The growth and production of monoclonal antibodies has been influenced by the development of new technologies. One major advance has been the development of non-secreting myeloma cell lines (Kearney et al., 1979). These non-secreting variants eliminate the dilution of antigen specific antibodies secreted by hybrid cells.

The development of serum-free media for growth of hybridomas has greatly facilitated the subsequent purification of monoclonal antibodies. Studies have shown that the addition of insulin and transferin to a serum-free hybridoma medium results in continuous growth of many antibody secreting cell lines (Chang, Steplewski and Koprowski, 1980).

Because yields of monoclonal antibodies in tissue culture medium are low (10–20 μg/ml), many investigators use antibodies generated in mouse ascites. Recent technology using hybridoma cells encapsulated in polymer microbeads (Nilsson et al., 1983) has greatly increased yields and allows for continuous cultures to be maintained.

Anti-idiotype antibodies

Monoclonal antibodies recognize a specific epitope consisting of linear or conformational regions of a molecule. The variable (binding) region of the monoclonal antibody molecule, the idiotype, then consists of a region complementary to the antigen epitope. The production of antibodies directed against another antibody molecule will result in some antibodies specific for the idiotypic region; these antibodies are called anti-idiotype antibodies (Marx, 1985). Anti-idiotype antibodies (either monoclonal or polyclonal) will have a binding region (idiotype) which is an image of the original antigen epitope and may be utilized in place of antigen in some cases for the identification or induction of antibody. Recently anti-idiotype antibodies have been used in diagnostic tests (Potocjnak et al., 1982) and as an immunogen to induce protective immunity against viral (Uytdehaag and Osterhaus, 1985) and parasitic infections (Sacks, Esser and Sher, 1982).

Application of monoclonal antibody technology in agricultural research

Applications of monoclonal antibody technology in the agricultural sciences may be loosely categorized as either (1) analytical, either biochemical or immunological, (2) diagnostic, or (3) therapeutic or prophylactic. Within these categories several main areas of research within the agricultural sciences may be identified.

Analytical applications of monoclonal antibodies

Monoclonal antibodies have been successfully used in the structural and functional analysis of a variety of agriculturally important proteins. A protein may be considered a series of antigenic regions or epitopes to which specific antibodies bind. Monoclonal antibodies, therefore, may be used to define the number of epitopes on a molecule by determining the number of distinct sites at which non-competitive antibody binding occurs. These methods have been used to identify distinct neutralizing epitopes on the HN glycoprotein of Newcastle disease virus (Iorio and Bratt, 1983), bovine leukemia virus glycoprotein GP51 (Bruck et al., 1982) and the capsid polypeptide VP1 of foot and mouth disease virus A12 (Robertson, Morgan and Moore, 1984), as well as antigenic regions on the phytochrome molecule (Silberman et al., 1985) and tobacco mosaic virus (van Reganmortel, 1984).

Monoclonal antibodies can be used to correlate biologic functions with specific regions on proteins and identify and map these antigenic regions of molecules following enzymatic or chemical degradation. For example, Bruck et al. (1984) demonstrated that monoclonal antibodies specific for neutralizing epitopes of bovine leukemia virus precipitated a proteolytic fragment of molecular weight 15 K from the virus envelop glycoprotein and showed that glycosylation was necessary for antibody binding. Similarly, Robertson et al. (1984) localized two neutralizing epitopes of foot and mouth disease virus within two specific amino acid sequences using competitive binding experiments with labeled monoclonal antibodies and competing virus and synthetic polypeptide fragments.

Monoclonal antibody binding can also shed light on the conformational structure of antigens. Antibody binding can define both continuous and discontinuous epitopes; continuous epitopes retain antibody binding properties with or without conformational integrity. Conversely, discontinuous or conformational epitopes do not bind antibody following denaturation (Van Regenmortel, 1984). Similarly, antibodies may define cryptic epitopes, those antigenic sequences not

exposed or available for antibodies in native conformation. During immune processing such regions may be exposed and appear to the host immune systems as foreign (antigenic). Such regions will only be recognized analytically by binding antibodies to denatured antigen.

Monoclonal antibodies have proven extremely useful for identification, quantitation, localization, and isolation of antigenic molecules of interest in the agricultural sciences. In the plant sciences, monoclonal antibodies have been used as immunocytochemical reagents to localize auxin carriers (Jacobs, 1984), phytochrome (Pratt, 1984), and cytokinins (Brandon, 1984).

In the animal sciences, monoclonal antibodies are proving to be extremely valuable as reagents for the identification and monitoring of the complex cellular interactions that regulate the immune response of livestock species. Recent studies have generated monoclonal antibodies that define specific subsets (T_h, T_s, T_c cells) of bovine (Davis, Perryman and McGuire, 1984), equine (Newman, Beegle and Antczak, 1984) and porcine (Peskovitz, Lunney and Sachs, 1984, 1985) leukocyte populations. Other studies have produced monoclonal antibodies that recognize specific classes and subclasses of bovine (Srikumaran et al. 1982) and porcine (Paul, Van Deusen and Mengling, 1985) immunoglobulins. The availability of these reagents will allow the dissection of the immune response to infectious agents in livestock species.

Monoclonal antibodies are currently being used as reagents to identify a subset of leukocyte antigens, gene products of the major histocompatability complex, as a method for studying the genetic regulation of immunity to disease in livestock. Using three independent swine lymphocyte antigen haplotypes in inbred miniature swine, Lunney (1984) have recently correlated these genetic differences with in vivo antibody and in vitro cellular responses to defined antigens. In the future correlation of differences in major histocompatibility complex loci in swine and other species with immune responses to purified antigens from infectious agents should provide information useful in the development of genetically resistant breeds of livestock.

Monoclonal antibodies are also being utilized as biochemical reagents for the affinity purification of biologically important molecules. In comparison to other methods such as chromatographic separations, monoclonal antibodies provide for a high degree of resolution (Chase, 1984), and are particularly useful for the recovery of recombinant proteins from fermentation products. The use of monoclonal antibodies as affinity reagents for the isolation of bovine somatotropin produced in *E. coli* has recently been demonstrated (Krivi and Rowold, 1984).

Diagnostic applications

Monoclonal antibodies are revolutionizing the fields of diagnosis and classification. Because of the property of specificity, monoclonal antibodies are often able to select between very closely related epitopes and hence closely related molecules or organisms. In addition to the properties of specificity, hybridoma produced reagents provide a source of homogeneous antibodies which can be used to standardize testing in all participating laboratories. The use of monoclonal antibodies in enzyme immunoassays and radioimmunoassays result in tests with a high degree of sensitivity.

Plant pathogens including viruses, fungi, bacteria, mycoplasma and nematodes are responsible for billions of dollars in economic losses in agricultural commodities annually. To prevent the spread of disease in plants or seed stock national and international certification and quarantine programs have been established. Antibody-based diagnostic tests have been applied to the detection of plant pathogens including potato viruses, ilarviruses, as well as bacterial, fungal and nematode diseases (Lankow et al., 1984); however, these serologic tests which use polyclonal sera have suffered limitations of specificity, reproducibility, and availability of reagents. Considerable emphasis has been placed on the production of monoclonal antibodies to be used in serology for plant disease diagnosis (Hsu, Jordan and Lawson, 1984). Recent studies have made available monoclonal antibodies with specificity for a variety of plant viruses including potyviruses (Hill, Hill and Durand, 1984) ilarviruses (Halk et al., 1984), alfalfa mosaic virus (Halk et al., 1984), the bacterial agents of citrus canker (Alverez, Benedict and Mizumoto, 1984) and potato ring rot (Magee, Beck and Ristow, 1984), and the fungi causing dwarf bunt disease in wheat (Banowetz, Trione and Krygier, 1984). Further use of monoclonal antibodies should greatly benefit the field of plant disease diagnostics.

In livestock, diagnosis of infectious disease requires the demonstration of the presence of the causative agent or in some cases the presence of a specific immune response to the causative agent. Monoclonal antibodies are rapidly replacing polyclonal antibodies for use in detecting infectious agents in host tissues. Where infectious agents are not detectable due to sequestering of antigens or presence in very low concentrations, detection may rely on a host antibody response to the organism. In this case monoclonal antibodies have been useful in identifying and isolating, by affinity chromatography, specific antigens for detecting antibody responses in binding assays (Gamble, 1984b).

In viral diagnosis, monoclonal antibodies have recently been used to identify the presence of bovine enteric coronavirus (Crouch, Raybould

and Acres, 1984) and antibody responses to infection with blue tongue virus (Anderson, 1984) and bovine leukemia virus (Mammerickx et al., 1984). For bacterial disease, monoclonal antibodies have been used to detect the presence of pili (Mills and Tietze, 1984) and enterotoxins (Thompson et al., 1984) produced by enterotoxigenic strains of *E. coli* causing bovine and porcine neonatal diarrhea and for lipopolysaccharide antigens differentiating strains of *Brucella* (Schurig, Hammerberg and Finkler, 1984; Quinn, Campbell and Phillips, 1984).

In the field of parasitology, monoclonal antibodies have been used for the differential diagnosis of coccidia species infecting poultry (Augustine and Danforth, 1984), and for the detection of trichinosis in swine (Gamble and Graham, 1984).

In addition to detecting pathogenic organisms in live animals, monoclonal antibodies are being used to identify and quantitate the presence of pathogenic contaminants (Mattingly, 1984) and chemical residues (Bishop and White, 1984) in food products. For example, an ELISA test has been developed using monoclonal antibodies which can detect all strains of *Salmonella* occurring in meat samples in half the time of conventional detection methods (Mattingly, 1984).

Therapeutic and prophylactic applications

Early in the history of monoclonal antibody technology there was great hope that science had found a "magic bullet" that would cure cancer. While such has not been the case, monoclonal antibodies have proven extremely useful in tumor imaging and diagnosis of other human diseases.

The prevention and treatment of animal disease has also benefited greatly from monoclonal antibody technology. The best examples of this technology have been the identification and isolation of antigens which can be used to vaccinate against infectious agents. Strategies for the use of monoclonal antibodies to identify protective antigens differ with respect to the type of infectious agent being studied. For example, delineation of immunogenic epitopes associated with viral diseases may be accomplished by demonstrating the role of monoclonal antibody binding in viral neutralization in vitro or by passive transfer or antibodies in vivo. Epitopes inducing specific neutralizing antibodies have thus been delineated for foot and mouth disease capsid polypeptides (Morgan et al., 1984; Robertson et al., 1984), Marek's disease virus glycoproteins (Ikuta et al., 1984) and polypeptides of bovine herpesvirus 1 (Collins et al., 1984). Control of *E. coli* diarrhea in neonatal calves and swine has relied largely on maternal vaccination. Recent studies have shown that oral administration of monoclonal antibodies specific

for the K99 pilus antigen of enteorotoxogenic *E. coli* passively protects animals in laboratory and field conditions (Morter, 1984; Sadowski, Wilson and Sherman, 1984). This targeted therapeutic use of monoclonal antibodies might obviate the need for universal vaccination of cows or sows.

Significant losses in livestock and poultry are caused by intracellular protozoan parasites including *Theileria* (East Coast fever in cattle), *Babesia* in cattle, and coccidia (*Eimeria* sp. in poultry). Monoclonal antibodies have been developed for *Theileria* (Dobbelaere et al., 1984; Musoke et al., 1984) and *Eimeria* (Danforth, 1983) which have anti-parasite activity in in vitro infectivity neutralization assays. These experiments suggest that antigens identified by these monoclonal antibodies may be effective for active immunization of livestock. Such has been the case for antigens of *Babesia bovis;* an antigen with molecular weight of 44 K isolated by affinity chromatography using monoclonal antibodies has been shown to induce significant immunity in cattle (Wright et al., 1983).

Nematode infections in livestock account for hundreds of millions of dollars annually in production losses and medication costs, however, little progress has been made in the search for immunological controls for these parasites. Because of the complexity of antigens and life cycles of these parasites, evaluation of specific antigens has proven difficult. In addition these parasites often act to alter or suppress the host immune response. Monoclonal antibody technology has provided a tool with which parasitologists may dissect the immune response by identifying single antigens and determining their role in immunity. Recent studies have shown that single helminth antigens, isolated using monoclonal antibodies, are capable of stimulating protective immunity against challenge infection with the parasitic nematode of swine *Trichinella spiralis* (Gamble, 1985; Silberstein and Despommier, 1984).

Conclusions

It is clear that the availability of monoclonal antibody technology has contributed significantly to solving problems in the agricultural sciences, as it has in other disciplines. With an increased need for improved agricultural technologies worldwide, the impact of this technology will undoubtedly grow and continue to contribute to the improvement of crop and animal quality and productivity.

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